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A new species of *Paramesotriton* (Caudata: Salamandridae) from Guizhou Province, China

XIAOMING GU[1, 5], RONGRONG CHEN[2], YINGZHOU TIAN[3], SONG LI[3] & JINGCHENG RAN[4]

[1] *School of Life Sciences, Guizhou Normal University, 180 N Baoshan Rd, Guiyang, Guizhou, China 550001.*
[2] *Molecular Biology Unit of King's College London, LondonUK*
[3] *Department of Biology, Liupanshui Normal College, Shuicheng, Guizhou China 553004*
[4] *The Administration Bureau of Maolan National Nature Preservation, Libo, Guizhou China 558400*
[5] *Corresponding author: E-mail: gxmswx@163.com*

Abstract

In this study, we describe a new species of salamander, *Paramesotriton maolanensis* **sp. n.**, from the Maolan National Nature Reserve, Libo County, Guizhou Province, China. The new species is placed in the genus *Paramesotriton* based on morphological characteristics and molecular data. It differs from all other members of the genus in a number of morphological characteristics, especially in its much larger body size, absence of granular warts from head and body, largely reduced external eyes and peculiar shape of epibranchia in hyoid apparatus. We examined the relationships of nuclear POMC haplotypes between and within the new species and six recognized species. POMC variation and published mitochondrial data suggested that the new species' closest known relatives are *P. longliensis*, *P. zhijinensis* and *P. caudopunctatu*s, and it should be placed into the *P. caudopunctatus* species group or subgenus *Allomesotriton*.

Key words: Caudata; Salamandridae; nuclear DNA; new species; *Paramesotriton maolanensis*

Introduction

The genus *Paramesotriton* (Caudata: Salamandridae), commonly known as the Asian warty newts, currently comprises eleven species: *P. caudopunctatu*s Liu and Hu, *P. chinensis* Gray, *P. deloustali* Bourret, *P. fuzhongensis* Wen, *P. guangxiensis* Huang, Tang and Tang, *P. hongkongensis* Myers and Leviton, *P. laoensis* Stuart and Papenfuss, *P. longliensis* Li, Tian, Gu and Xiong, *P. zhijinensis* Li, Tian and Gu, *P. ermizhaoi* Wu, Rovito, Papenfuss and Hanken, and *P. yunwuensis* Wu, Jiang and Hanken. The last four species were described recently (Li *et al*. 2008 a, b; Wu *et al.* 2009, 2010).

One species from Laos initially described in *Paramesotriton* as *P. laoensis* is remarkably different, with a bright dorsal coloration (Stuart & Papenfuss 2002). Weisrock *et al*. (2006) and Zhang *et al*. (2008) suggested that *P. laoensis* constitutes a different lineage from *Paramesotriton,* and a new monotypic genus, *Laotriton,* was proposed for this species (Dubois & Raffaélli 2009).This proposal was followed by Frost (2011) in the Amphibian Species of the World database (ASW), and in Amphibiaweb (http://www.amphibiaweb.org, accessed November 2011).

Sparreboom (1983) reported the morphological features, reproduction and egg-laying behavior of *P. caudopunctatus*. Based on morphological and osteological characteristics, Freytag (1983) considered that *P. caudopunctatus* is distinct from congeners and proposed a new genus, *Allomesotriton*, for this species. However, Freytag's taxonomic change proposal has not been accepted widely. Pang et al. (1992), Fei et al. (2006), and Dubois and Raffalli (2009) considered *Allomesotriton* as a species group or a subgenus within *Paramesotriton.* Recent studies suggested that the *P. chinensis* species group or subgenus *Paramesotriton* includes *P. chinensis*, *P. fuzhongensis*, *P. guangxiensis*, *P. hongkongensis, P. deloustali, P. ermizhaoi* and *P. yunwuensis*; the *P. caudopunctatus* species group or subgenus *Allomesotriton* comprises *P. caudopunctatus*, *P. zhijinensis*, *P. longliensis* and a newly discovered population (*P. maolanensis* **sp. n.**, the new species described in the paper, for

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which we already use here the new name formally introduced below) which is native to Libo county of Guizhou Province; and the monophyly of the genus and of the two species groups (subgenera) has been demonstrated (Wu *et al*. 2010; Gu *et al.* 2011).

Guizhou is a province in the southwestern mountainous region of China and holds rich amphibian faunal diversity. Three *Paramesotriton* species, *P. caudopunctatus* (Liu and Hu 1973), *P. zhijinensis* and *P. longliensis* (Li *et al.* 2008. a,b), were described from specimens captured in this province. *P. maolanensis* **sp. n.** also occurs in the south of Guizhou (Fig. 1). These salamanders are morphologically different from all other known *Paramesotriton* species. We acquired five specimens from this population in Guizhou Province and have previously explored its phylogenetic relationship to other *Paramesotriton* species based on mitochondrial DNA sequence data (3189 bp), suggesting that *P. maolanensis* **sp. n.** is sister to *P. longliensis* (Gu *et al.* 2011). Herein we combine evidences from nuclear DNA, mitochondrial DNA and morphology to determine its identity as new species, and to provide its formal description.

Material and methods

Five specimens of *P. maolanensis* **sp. n.**were sampled in July 2006 and July 2008 from the Maolan National Nature Reserve, Libo County, Guizhou Province, P. R. China. Fresh muscle and liver tissues were taken from the specimens and preserved in 90% ethanol used for genetic analyses. All specimens were stored in 75% ethanol, and these specimens and tissue samples used in this study were deposited in the School of Life Sciences, Guizhou Normal University, Guiyang, Guizhou P. R. China.

The preserved specimens were measured with calipers to the nearest 0.1 mm. Measurements of adults were followed Fei *et al*. (2005). The following abbreviations were used: TOL, total length; SVL, snout-vent length measured from tip of snout to anterior edge of vent; HL, head length measured from posterior edge of left parotoid to snout tip; HW, maximum head width; HH, head high; SL, snout length; IO, interocular distance measured from anterior corner of eye; ED, eye diameter; IN, internostril distance; AX, distance between axillae along right body side; TL, tail length; TW, tail width; TH, tail high; FLL, forelimb length; HLL, hindlimb length.

The hyoid apparatuses and skulls of two specimens were prepared and compared with seven other species of the genus (Pang *et al.*1992, Li *et al.* 2008, a, b). Since we could not access to voucher specimens of three species, *P. deloustali*, *P. ermizhaoi* and *P. yunwuensis,* we compared morphological characteristics to original descriptions for holotypes, and also images and species diagnoses on the Amphibiaweb (http://www. amphibiaweb.org/lists/Salamandridae.shtml) for these species.

The detailed phylogenetic relationships of *P. maolanensis* **sp. n.** to other *Paramesotriton* species based on mitochondrial DNA sequence data (3189 bp) have been published in Gu *et al.* (2011). Here we used a gene that encodes part of the proopiomelanocortin protein (POMC), a single-copy nuclear marker, to validate the relationships. This gene has been widely used in resolving relationships among Caudata species (Vieites *et al.* 2007, Stuart *et al.* 2010). In addition to *P. maolanensis* **sp. n.**, six other species of *Paramesotriton* were examined in this study. They are *P. chinensis*, *P. guangxiensis*, *P. fuzhongensis*, *P. caudopunctatus* (including Leishan, Jiangkou and Youyang populations), *P. zhijinensis*, and *P. longliensis* (including Longli and Suiyang populations) (see Fig 1 for sampling localities and Table 1 for number of samples, respectively).

Genomic DNA was extracted from the muscle tissues using the standard phenol-chloroform method. A fragment of POMC gene was amplified by standard PCR (one cycle of 94 °C 5 min, 35 cycles of 94 °C 45s, 48 °C 30s, 72 °C 1 min, one cycle of 72 °C 10 min) using the primers POMC_DRV_F3 and POMC_DRV_R1 (Vieites *et al.* 2007). The PCR products were purified and sequenced in both directions using the same primer pairs at Shanghai DNA BioTechnologies Co, Ltd (Shanghai, China).

The POMC gene fragment was sequenced in both directions in order to verify possible heterozygous sites. All sequences were checked for their quality visually in the program Clustal X 1.81 (Thompson. *et al.* 1997) and aligned manually. All sequences were deposited in Genbank; see Table 1 for sample and Genbank accession numbers, and localities. We used the software TCS v1.21 (Clement *et al.* 2000) to reconstruct a haplotype network of POMC haplotypes.

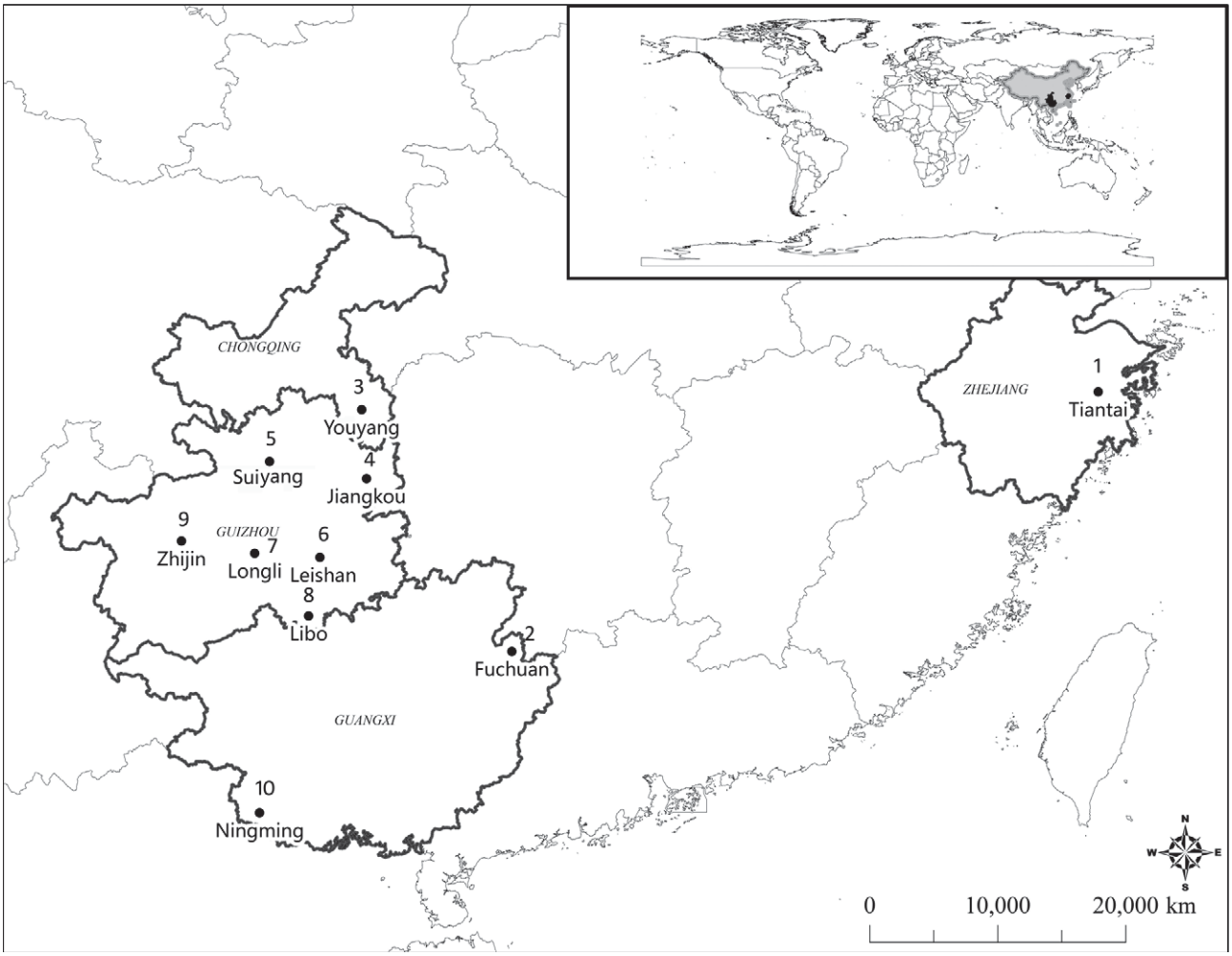


FIGURE 1. Map showing the distribution of the species of *Paramesotriton* used in this study. 1: *P. chinensis*; 2: *P. fuzhongensis*; 3: *P. caudopunctatus* (Youyang population); 4: *P.caudopunctatus* (Jangkou population); 5: *P. longliensis* (Suiyang population); 6: *P.caudopunctatus* (Leishan population, topotype); 7: *P. longliensis* (topotype); 8: *P. maolanensis* **sp. n.**; 9:*P. zhijinensis*; 10: *P. guangxiensis*. For information on species, see Table 1.

TABLE 1. *Paramesotriton* species sampling for samples and the haplotypes of the nuclear POMC gene fragment used in this study. GZNU=Guizhou Normal University (Guiyang, China).

Species	Locality	Voucher Specimen No.	GenBank Accession No.	Haplotypes
P. maolanensis **sp. n.**	Libo, Guizhou, China	GZNU2006030003	JQ680398	H1
P. maolanensis **sp. n.**	Libo, Guizhou, China	GZNU2006030004	JQ680399	
P. maolanensis **sp. n.**	Libo, Guizhou, China	GZNU2006030005	JQ680400	
P. longliensis	Longli, Guizhou, China	GZNU20070421001	JQ680392	H2
P. longliensis	Longli, Guizhou, China	GZNU20070421002	JQ680393	
P. longliensis	Suiyang, Guizhou, China	GZNU2011111001	JQ680394	
P. longliensis	Suiyang, Guizhou, China	GZNU2011111002	JQ680395	
P. zhijinensis	Zhijin, Guihzou, China	GZNU20070415002	JQ680396	H3
P. zhijinensis	Zhijin, Guihzou, China	GZNU20070415001	JQ680397	
P. caudopunctatus	Leishan, Guizhou, China	GZNU200904252	JQ680389	H4
P. caudopunctatus	Leishan, Guizhou, China	GZNU200904251	JQ680390	

..... continued on the next page

TABLE 1. (Continued)

Species	Locality	Voucher Specimen No.	GenBank Accession No.	Haplotypes
P. caudopunctatus	Leishan, Guizhou, China	GZNU2007072005	JQ680391	
P. caudopunctatus	Jiankou, Guizhou, China	GZNU2007071004	JQ680385	H5
P. caudopunctatus	Jiankou, Guizhou, China	GZNU2007071002	JQ680386	
P. caudopunctatus	Youyang, Chongqing, China	GZNU08072602	JQ680387	
P. caudopunctatus	Youyang, Chongqing, China	GZNU08072603	JQ680388	
P. guangxiensis	Ningming, Guangxi, China	GZNU2006001	JQ680379	H6
P. guangxiensis	Ningming, Guangxi, China	GZNU2006002	JQ680380	
P. fuzhongensis	Fuchuan, Guangxi, China	GZNU2007052001	JQ680381	H7
P. fuzhongensis	Fuchuan, Guangxi, China	GZNU2007052002	JQ680382	
P. fuzhongensis	Fuchuan, Guangxi, China	GZNU2006067003	JQ680383	
P. chinensis	Tiantai, Zhejiang, China	GZNU200806087	JQ680384	H8

Results

Molecular phylogenetics

The fragments consisted of 460 nucleotides after alignment and exclusion of incomplete positions at the beginning and end of sequences. No heterozygotes were detected. The analyzed fragments correspond to nucleotide sites 222–681 of the POMC segment of *Cynops pyrrhogaster* (NCBI Reference Sequence: AB572298.1). The POMC sequences showed much lower variability than the mitochondrial sequences. There were 8 parsimony informative sites out of a total of 10 variable nucleotide positions in the POMC alignment including *P. maolanensis* **sp. n.**, *P. chinensis*, *P. guangxiensis*, *P. fuzhongensis*, *P. caudopunctatus*, *P. zhijinensis* and *P. longliensis* (9 parsimony informative sites out of a total of 16 variable nucleotide positions if *Cynops pyrrhogaster* was included).

The nucleotide differences separating *P. maolanensis* **sp. n.** from *P. zhijinensis* and *P. longliensis*, and separating *P. zhijinensis* from *P. longliensis* all are 2.0 (0.43% *p*-distance). The nucleotide differences separated *P. maolanensis* **sp. n.** from *P. caudopunctatus*, (Leishan population) and *P. caudopunctatu* (Youyang and Jiangkou populations) are 2 (0.43% *p*-distance) and 3 (0.65% *p*-distance), respectively. The nucleotide differences separated *P. maolanensis* **sp. n.** from *P. chinensis, P. fuzhongensis* are much higher, i.e., 5 (1.09% *p*-distance) and 4 (0.87% *p*-distance), respectively.

Eight different haplotypes were distinguished, and they were partitioned into two branches based on two mutations: the first branch comprises *P. maolanensis* **sp. n.** (H1), *P. longliensis* (H2), *P. zhijinensis* (H3) and *P. caudopunctatus* (H4 and H5); the second branch includes *P. guangxiensis* (H6), *P. fuzhongensis* (H7) and *P. chinensis* (H8). H1 groups three specimens of *P. maolanensis* **sp. n.**, H2 groups four specimens of *P. longliensis* (two from Longli and Suiyang, respectively), H3 groups two specimens of *P. zhijinensis,* H4 groups three specimens of *P. caudopunctatus* (from Leishan), H5 (separated by a single mutation from H4) groups four specimens of *P. caudopunctatus* (two from Jiangkou and Youyang, respectively), H6 groups two specimens of *P. guangxiensis,* H7 (separated by a single mutation from H6) groups three specimens of *P. fuzhongensis*, and H8 (separated by two mutations from H6) groups one specimen of *P. chinensis*. All seven species could be clearly separated in the haplotype network (Fig. 2).

P. maolanensis **sp. n.**, *P. longliensis*, *P. zhijinensis* and *P. caudopunctatus* are assigned to *P. caudopunctatus* group or the subgenus *Allomesotriton* in the phylogeny based on the mitochondrial DNA sequence data. The phylogenetic relationship among *P. longliensis*, *P. zhijinensis* and *P. maolanensis* **sp. n.** is [*P. zhijinensis* (*P. longliensis*, *P. maolanensis* **sp. n.**)]. Taken together, this clade is the sister group to *P. caudopunctatus*. The mitochondrial DNA sequence data indicate only a low mitochondrial divergence and thus possibly a young split divergence between *P. maolanensis* **sp. n.** and *P. longliensis* (Fig. 3. Gu *et al.* 2011).

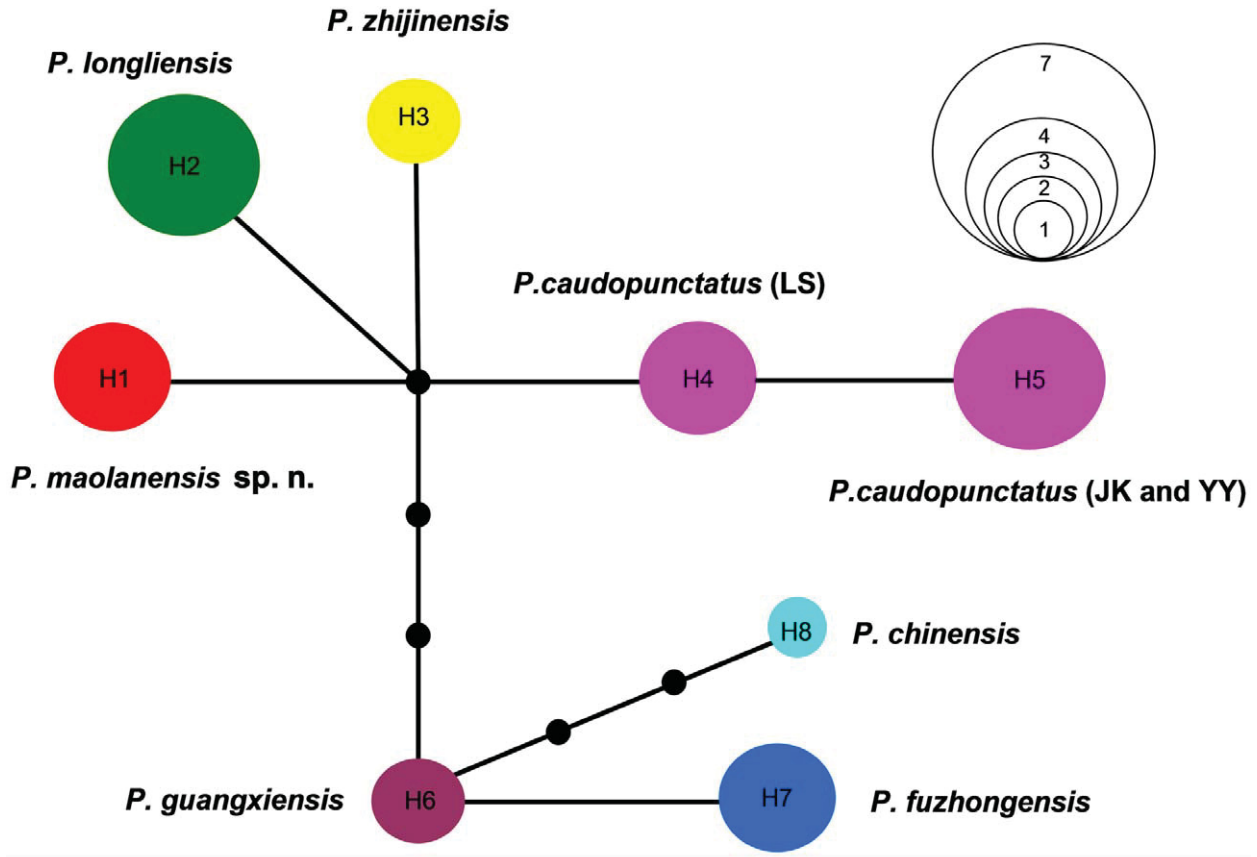
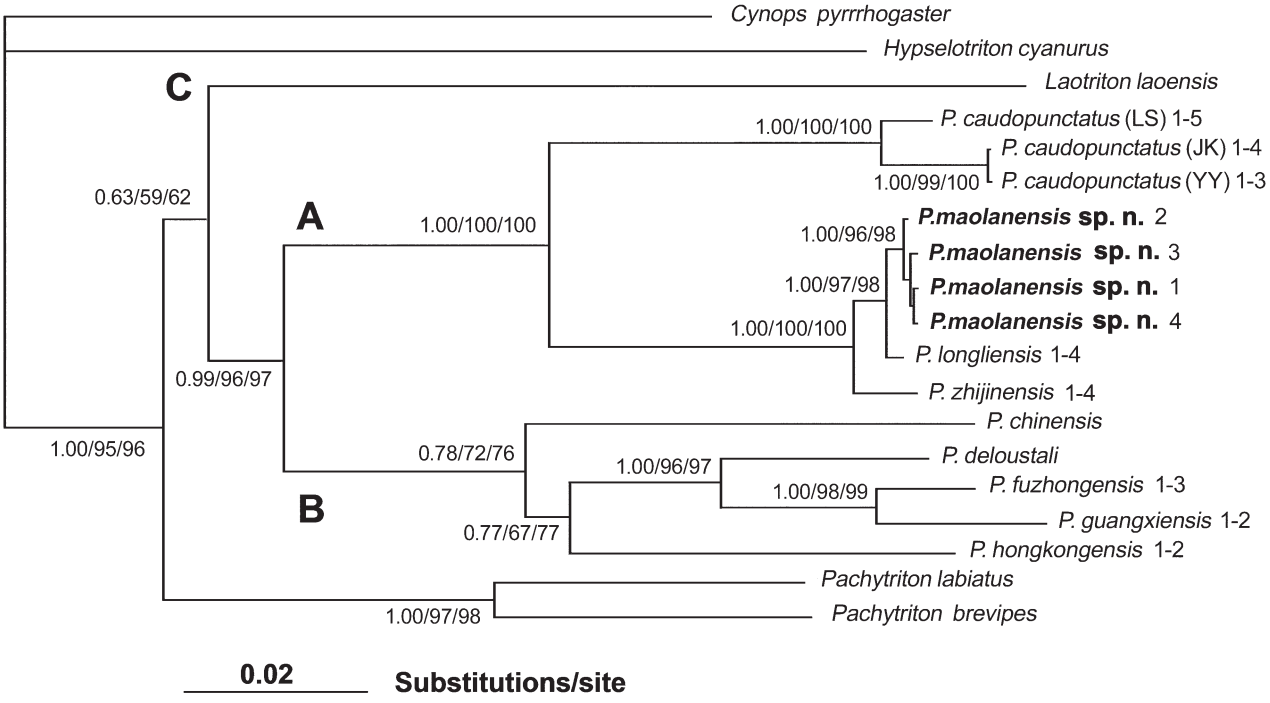


FIGURE 2. Haplotype network based on variation in POMC sequences in seven *Paramesotriton* species. H1–H8 denote major haplotypes, the size of circles is proportional to the number of individuals (all were homozygotes) bearing a certain haplotype (H8: 1 individual; H3 and H6: 2 individuals; H1, H4 and H7: 3 individual; H2 and H5: 4 individuals).



FIGUER 3. Phylogenetic relationships of extant *Paramesotriton* species (except *P. ermizhaoi*) derived from the maximum parsimony, partitioned maximum likelihood and Bayesian inference analyses of the mitochondrial DNA sequence data (3189 sites, reproduced from Gu *et al*. 2011). This is a simplified figure, *P.* sp in the original figuer is referred to as. *P. maolanensis* **sp. n.** here. Left numbers along branches represent weighted maximum parsimony bootstrap values, middle numbers represent partitioned maximum likelihood bootstrap values and right numbers represent Bayesian posterior probabilities. Branch lengths were estimated by Bayesian inference analysis. LS, JK and YY in bracket after specific name represent Leishan, Jiangkou and Youyang populations of the species, respectively; the numbers after specific name represent the serial number of specimens for the species.

Systematics

P. maolanensis **sp. n.** can be distinguished from other *Paramesotriton* species by its comparatively much larger body size, absent granular warts from head and body, relative degraded external eyes (vestigial lenses), the presence of huge horn protuberances on the upper laterals after head and three morphological traits in hyoid apparatus: (1) short basihyoid, (2) second ceratobranchial bone is bone, not cartilage, (3) the front end of epibranchial bone diverges and presents a"Y" shape to connect the first, second ceratobranchial bones, respectively.

The new species has an obviously larger body size than other *Paramesotriton* species. Its TOL is 177–208 mm while the TOL of the previously known largest species, *P. deloustali*, in this genus is 160–200 mm and the TOL ranges from 94 to 186 mm for other species. The new species further differs from all other *Paramesotriton* species from other aspects: 1) granular warts absent from head and body (granular warts presenting on head and body in other described species of *Paramesotriton*) and 2) relative degraded external eyes (under natural conditions the upper and lower eyelids of the new species close, but, the upper and lower eyelids do not cover their well developed lenses in other described *Paramesotriton* species) (Fig. 6 A, B and C).

The presence of horn protuberances on the upper laterals after head is shared by only two species: the new species and *P. longliensis*. However, the size of the horn protuberances of the new species is distinctly larger than that of *P. longliensis*(5.5–6.7 mm versus 2.2–3.3mm).

Regarding the hyoid apparatus, the basihyoid lengths for the new species, *P. zhijinensis*, *P. longliensis* and *P. caudopunctatus* are clearly shorter than those of *P. chinensis, P. hongkongensis, P. fuzhongensis* and *P. guangxiensis* (Pang *et al*. 1992). A shorter basihyoid is possibly a synapomorphy of *P. caudopunctatus* species group or the subgenus *Allomesotriton.* The second ceratobranchial bone is ossified for the new species. It is obviously different from those of *P. longliensis* and *P. zhijinensis,* which are all cartilage. The front ends of the epihyal and the epibranchial bone of the new species diverge and form a "Y" shape. This trait is also obviously different from *P. longliensis* and *P. zhijinensis* in which the front ends of the two bones does not present a conspicuous "Y" shape. Only the front ends of the epibranchial bone present a"Y" shape for *P. caudopunctatus* (Fig. 7 A, B, C & D).

In addition, skulls of the new species*, P. zhijinensis, P. longliensis* and *P. caudopunctatus* are all long and narrow. HW/HLs of them are 0.76, 0.79, 0.75 and 0.75, respectively. But HW/HLs of *P. chinensis*, *P. hongkongensis*, *P. fuzhongensis*, *P. guangxiensis* and *P. yunwuensis* are all greater than 0.80, (except for *P. ermizhaoi*, HW/HL = 0.78) (Pang *et al*. 1992; Li *et al.* 2008. a, b; Wu *et al*. 2009, 2010).

In conclusion, molecular phylogenetic analyses and morphological examination support the recognition of the newly discovered population of *Paramesotriton* from south Guizhou as a distinct species in the *P. caudopunctatus* species group or the subgenus *Allomesotriton*. We describe the new species below.

Paramesotriton maolanensis, new species

(Fig. 4 A–F, Table 2)

Holotype: GZNU 2006030001, an adult male from Wengang, 25°40'N 107°53'E, 817m a.s.l., Libo County, Guizhou Province, P. R. China; collected by Hanji Qin in July, 2008.

Paratypes: adults: GZNU2006030003-5, GZNU2008070001, 4 specimens in total collected in July, 2006 and July, 2008 at the same locality as the holotype.

Diagnosis: *Paramesotriton maolanensis* **sp. n.** is assigned to the genus *Paramesotriton* because of its glandular ridge on head side, premaxillary single pieces, long nose protuberance separated left and right nasal bones, maxilla shorter not touching quadrate bone, and laterally compressed tail. This species can be distinguished from all other species of *Paramesotriton* by the following combination of characteristics: relatively smooth skin, absent granular warts from head and body; large body size (the largest species among the genus *Paramesotriton*), especially in the female, TOL 177.4–192.0 mm for male and 197.4–207.8 mm for female; external eyes relative degraded; short skin pleat swelled dorsomidmost ridges, forming buff dorsal fin pleat from occiput to end of tail; dorsolateral stripes indistinct, obviously different from congeners.

TABLE 2. Linear measurements of *Paramesotriton maolanensis* **sp. n.**. See text for abbreviations. Unit: mm.

Measurement	Males (N=3)				Females (N=2)		
	Holotype GZNU 2006030001	Paratype GZNU 2006030003	Paratype GZNU 2006030004	Mean ± SE	Paratype GZNU 2006030005	Paratype GZNU 2008070001	Mean
TOL	187.1	177.4	192.0	184.7± 4.3	197.4	207.8	202.6
SVL	94.2	89.7	98.5	94.1 ± 2.5	112.5	116.8	114.7
HL	34.2	32.3	34.7	33.7 ± 0.7	35.6	37.6	36.6
HW	25.2	24.4	26.5	25.4 ± 0.6	26.7	29.1	27.9
HH	14.4	13.1	16.2	14.6 ±0.9	13.5	16.8	15.2
SL	9.2	8.4	9.8	9.1 ± 0.4	9.2	10.3	9.8
IO	8.7	8.1	9.0	8.6 ± 0.3	9.0	10.4	9.7
ED	4.9	4.6	5.0	4.8 ± 0.1	4.9	5.5	5.2
TL	86.7	81.8	90.5	86.3 ± 2.5	87.7	94.5	91.1
TW	11.2	9.5	12.1	10.9 ± 0.8	10.8	13.5	12.2
TH	15.2	14.8	16.0	15.3 ± 0.4	11.7	13.5	12.6
FLL	29.1	26.1	31.8	29.0 ± 1.7	29.2	34.6	31.9
HLL	33.1	30.3	35.3	32.9 ± 1.5	32.9	38.0	35.5
IN	6.1	5.8	6.6	6.2 ± 0.2	5.9	6.4	6.2
AX	38.4	37.0	41.6	39.0 ± 1.4	53.6	57.1	55.4

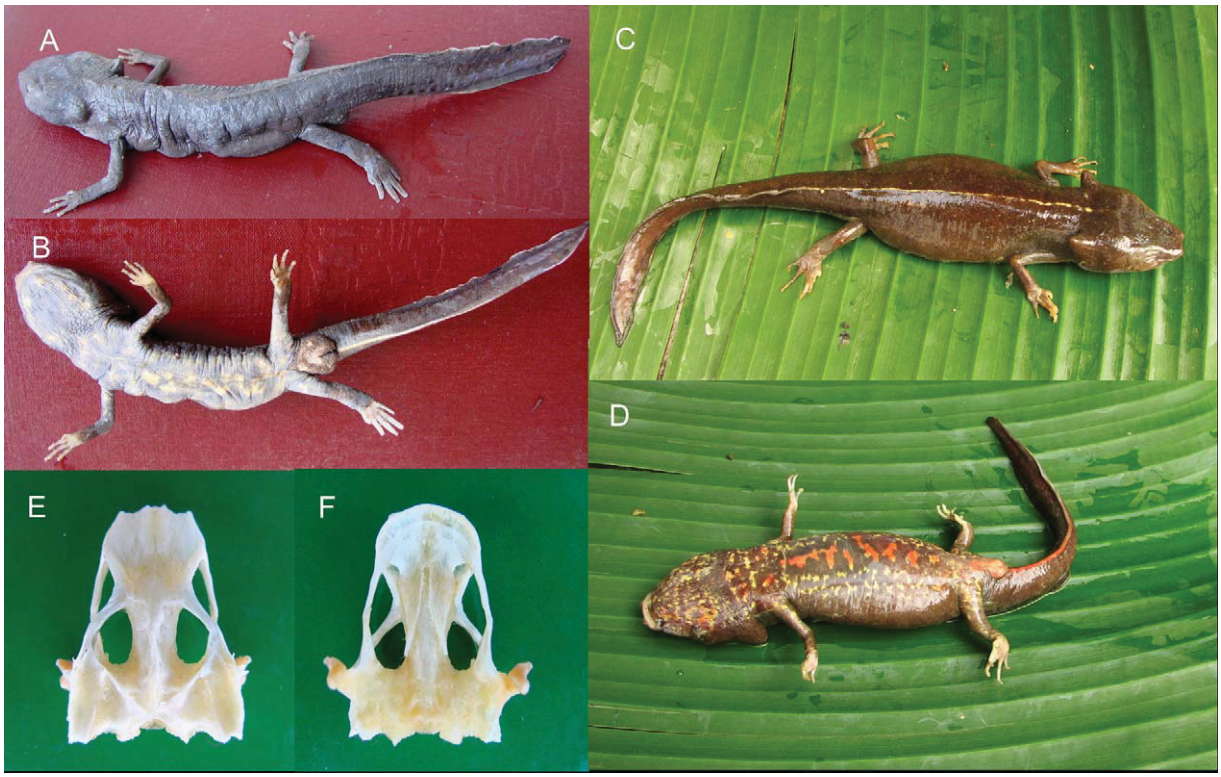


FIGURE 4. A & B. Dorsal, lateral view and ventral, lateral view of *Paramesotriton maolanensis* **sp. n.** (GZNU 2006030001, holotype). C & D. Dorsal view and ventral, lateral view of *P. maolanensis* **sp. n.** (GZNU2008070001, paratype). E & F. Dorsal view and ventral view of skull of *P. maolanensis* **sp. n**. (GZNU2006030003, paratype).

P. maolanensis **sp. n.** further differs from *P. longliensis* and *P. zhijinensis* by its ceratobranchial being bone (not cartilage) and the epibranchial bone diverges forming a "Y" shape, and two ends of the "Y" shape joining the first, second ceratobranchial bones, respectively, in hyoid apparatus.

Description of the holotype: The specimen is in a good state of preservation. Measurements are given in Table 2. This is a large newt, TOL is 187.1 mm. Head length distinctly longer than head width. Snout length obviously longer than the diameter of eye. Head strongly oblique in profile. Skull broad with maxillaries oriented angular to body axis. Snout short, truncated, extending beyond lower lip; snout arris evident. Nostrils locate on the tip of snout. External eyes relative degraded. Lip pleat developed, extending from lower part of eye to tip of snout. Tongue in elliptical shape and both lateral sides dissociatived. Retral-limbs longer and sturdier than forelimbs. Tip of finger reaches tip of snout when forelimbs keep close to body and extend forward, palm part and sole part superpose one another when forelimb and retral-limb keep close to body and confrontative. Four fingers and five toes, keratose theca on tips of figures and toes,whithout velum and webbing. The third and fourth toes almost equal in length, both whithout inside and outside palm-sole tubers. Dorsomidmost ridge buff forming dorsal fin pleat on tail; ventral tail fin salmon pink, extending from the posterior edge of vent to end of tail. Testicle globose and dimidiate at each side. One premaxilla with tiny teeth on the upper and lower jaws. Vomerine teeth in ' ∧ ' pattern. Front edges of dentition rendezvoused between two inner nares. Front end of the epibranchial bone of hyoid apparatus in "Y" shape joining the first, second ceratobranchials, respectively. The second ceratobranchial is bone. Apophysis of vent of the male large and low; anus cleft a vertical thread with 6–7 mm with a pair of developed glands in inside; the counter part of the female small and high; anus cleft small and oval without gland in inside.

Color of holotype in life: Body is brown-black; one tubercular dorsal ridges with non-continuous yellow mottling. There are large, irregular orange-red spots on venter, chin, some smaller and yellow spots interspersed on the laterals of venter and chin. Palm-sole part surface is hoar.

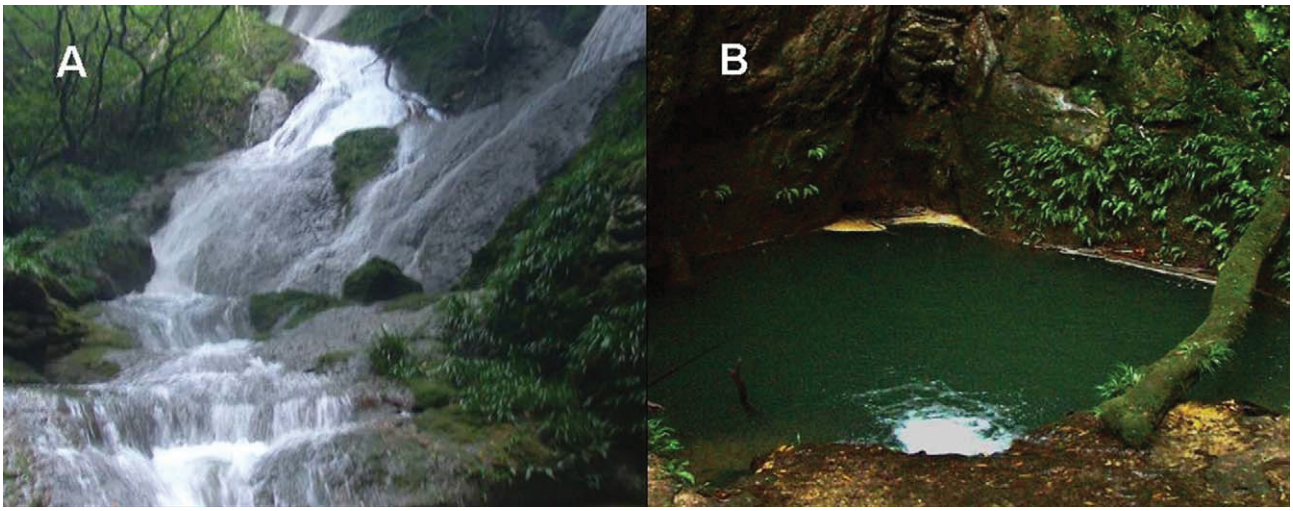


FIGURE 5. A. One of the two rivulets that flow into the deep pool; B. The deep pool where *P.maolanensis* **sp. n.** were collected.

Color in holotype in preservative: In 70% ethanol the dorsal brown-black faded to black, yellow dorsal ridge faded to white, or fade away; ventral orange-red and yellow spots faded to yellow and buff, both palm-sole part and finger-toe ventral surface buff.

Variation: The stripes of dorsal ridge of adults are variable, sometimes indistinct. Ventral spots vary in shape and arrangement. All other adult morphological characteristics conform to those of the holotype.

Distribution and Habitat: The Maolan National Nature Reserve locates in Libo County of the south on Guizhou Province which is next to Nandan County of Guangxi Province. The karst forest of the reserve, survived the same latitude in the world, is a large centralized distribution, relatively stable and native forest ecosystems. There are many rare and endangered wild animals and plants in the reserve. All specimens of *P. maolanensis* **sp. n.** are found in a 60 m^2 deep pool which is surrounded by lush vegetation in the reserve .Two rivulets from the springs and caves (the lengths are about 0.3 km) disembogue water into the deep pool, but the water level of the pool keeps steady which implies the deep pool communicates with underground river (Fig. 5). The clear water in the pool is

warm with a temperature of about 17°C year round. The salamanders usually move into the deep and bottom of the pool and are difficult to be found. They contingently jump out water during the floods. In addition, the pool supports large quantities of aquicolous hexapods, shrimps, crabs and fishes.

FIGURE 6. The external eyes of *P. maolanensis* **sp. n.**, *P. zhijinensis* and *P. longliensis* under natural conditions. A. *P. maolanensis* **sp. n.** (GZNU2008070001, paratype), the upper and lower eyelids is occlusive. B and C. *P. longliensis* and *P. zhijinensis* (GZNU2012070006 and GZNU2012070002), the upper and lower eyelids do not cover well developed lens.

Etymology: The specific name *maolanensis* is an adjective referring to its known distribution in the Maolan National Nature Reserve of Libo County, Guizhou Province, China.

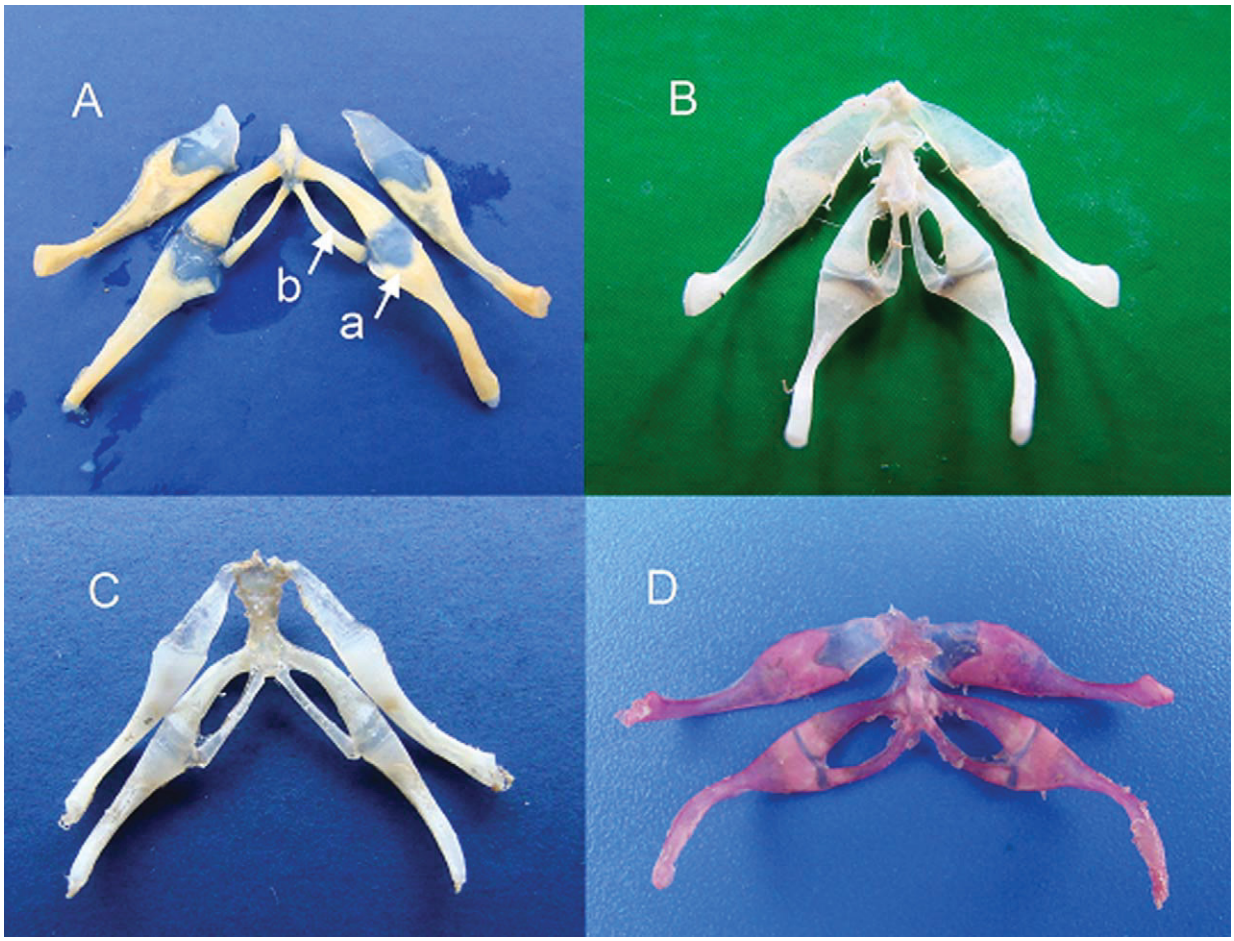


FIGURE 7. Structure of hyoid apparatus of *P. maolanensis* **sp. n.** and other three species in subgenus *Allomesotriton*. A. *P. maolanensis* **sp. n.** (GZNU 2006030004, paratype): a. epibranchial bone present a "Y"shape, b. the second ceratobranchial is bone. B, C & D. *P. longliensis* (LPSNC0705002), *P.zhijinensis* (LPSNC 20110802) and *P. caudopunctatus* (GZNU 2007071002). The differences between *P. maolanensis* **sp. n.** and these three species are given in the text above.

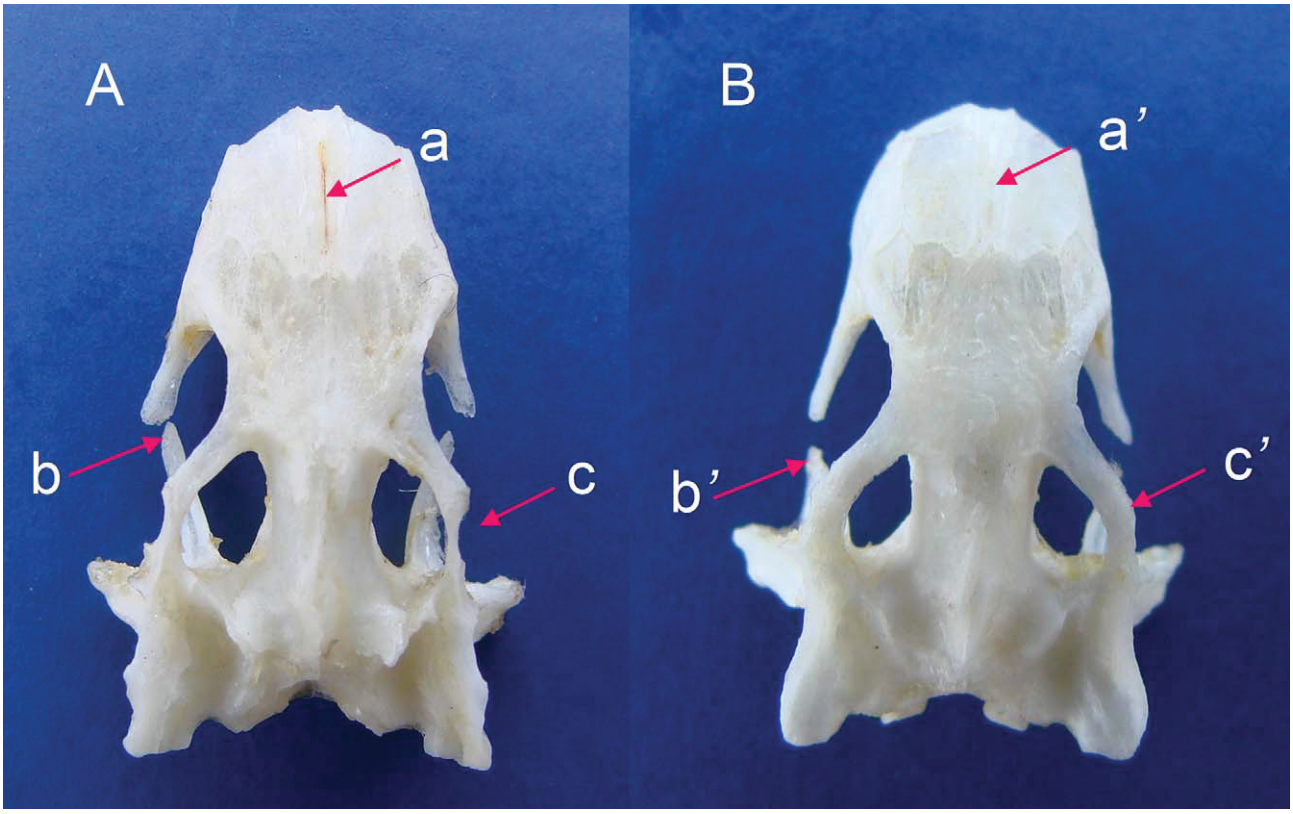


FIGURE 8. Borsal view of skull of *P. caudopunctatus* (Youyang) and *P. caudopunctatus* (Youyang) and *P. caudopunctatus* (Leishan). A and B are *P. caudopunctatus* (Jiangkou, LPSNC20110721, ♂) and *P. caudopunctatus* (Leishan, LPSNC2007072005, ♂), respectively. a and a', suture in the middle of nose protrusion; b and b', frontal branch of pterygoid. c and c', squamosal of forehead-squamosal arc. The differences between *P. caudopunctatus* (Youyang) and *P. caudopunctatus* (Leishan) are given in the text above.

Discussion

Despite a rather low differentiation in the mtDNA data, the absence of POMC haplotype sharing between *P. maolanensis* **sp. n.** and its sister group *P. longliensis* confirms that recent gene flow between these species is restricted and absent, and together with the strong morphological divergences clearly suggests *P. maolanensis* **sp. n.** representing a distinct species. It is noteworthy that four specimens of *P. caudopunctatus* (Jiangkou and Youyang populations, H5) could be clearly separated by a single mutation from *P. caudopunctatus* (Leishan population, H4) in the POMC haplotype network, and seven specimens of *P. caudopunctatus* (Jiangkou and Youyang), including this four specimens in the POMC haplotype network, form a monophyletic group in the mtDNA analysis (Fig. 3, Gu *et al.* 2011). Further more, Jiangkou and Youyang populations are different from Leishan population (topotype) in a number of morphological characteristics, especially skull characteristics: the sutures in the middle of nose protrusion of Jiangkou and Youyang populations are deeper than those of the topotype, the squamosals of forehead-squamosal arc of Jiangkou and Youyang populations reach vertically, and the fronts are crude and the backs are fine from dorsal view, but those of the topotype reach horizontally, and the thicknesses of the fronts and the backs are the same from dorsal view, and frontal branchs of the pterygoid of Jiangkou and Youyang populations are longer and arrive at the posterior edge of maxillary, but those of the topotype are shorter and don't arrive at the posterior edge of maxillary (Fig. 8). These suggest that these salamanders from Jiangkou and Youyang may be a new species.

P. maolanensis **sp. n.**, *P. zhijinensis* and *P. longliensis* all live in the environment close related to the karst cave and underground rivers. *P. maolanensis* **sp. n.** lives in underground river in caves while *P. zhijinensis* lives in the pool of which water flows from springs and *P. longliensis* lives in the stream originating from springs. The pool and stream of the latter two species are near to springs. Because of adaptation to life in underground river in the

cave, the external eyes of *P. maolanensis* **sp. n.** are relative reduced (vestigial lens). The species may be a blind subterranean species without functional image-forming eyes. *P. zhijinensis* and *P. longliensis* are usually seen at the surface of water. These two surface species have well developed crystalline lenses. Further study is needed to explore whether there are other subterranean species similar to *P. maolanensis* **sp. n.** which has adapted to life in the underground rivers from caves near its habitat. *P. caudopunctatu*s and other *P. chinensis* species group or the subgenus *Paramesotriton* species all live in streams not directly related to the caves and underground rivers and have external eyes with well developed crystalline lenses.

Most adult specimens of *P. zhijinensis* exhibit vestigal gills and gill filaments which is a characteristic of paedomorphic salamanders. The species *P. longliensis* does not possess such a characteristic (Li *et al*. 2008 a, b). *P. maolanensis* **sp. n.** also does not have this paedomorphic characteristic. Given that *P. maolanensis* **sp. n.** in head shape and eye reduction is roughly similar to other paedomorphic salamanders such as *Eurycea waterlooensis*, adults of which have gills and lack external eyes (there are no lenses) (Hillis *et al*., 2001), it is likely to be a blind subterranean species adapted to life in underground river in the cave. We could however not conclude that *P. maolanensis* **sp. n.** is a paedomorphic salamandrid because we did not find obvious paedomorphic characteristics similar to *P. zhijinensis* and *Eurycea waterlooensis* for *P. maolanensis* **sp. n.**.

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References

Amphibiaweb. www.amphibiaweb.org

Clement, M., Posada, D. & Crandall, K. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657–1660.

Dubois, A & Raffaelli, J. (2009) A new ergotaxonomy of the family Salamandridae Goldfuss, 1820 (Amphibia, Urodela). *Alytes*, 26 (1–4), 1–85.

Fei, L., Ye, C.Y., Jiang, J.P., Xie, F. & Huang, Y.Z. (2005) *An Illustrated Key to Chinese Amphibians*. Sichuan Publishing Group and Sichuan Publishing House of Science and Technology, Chengdu, China. 3–6. (in Chinese)

Fei, L., Hu S.Q., Ye, C.Y., & Huang Y.Z. (2006) *Fauna Sinica.Amphibia V01.1.General accounts of Amphibia, Gymnophiona and Urodela.* Science Press, Beijing, China, pp. 253–313. (in Chinese)

Frost, D.R. (2011) Amphibian Species of the World: an Online Reference. Version 5.5 (31 January, 2011). Electronic Database accessible at http://research.amnh.org/vz/herpetology/amphibia/American Museum of Natural History, New York, USA.

Freytag, G.E. (1983) Zur systematischen Stellung von *Trituroides caudopunctatus* (Liu & Hu, 1973). Morphometrische und röntgenanatomische Befunde (Amphibia, Caudata, Salamandridae). *Zoologische Abhandlungen Staatliches Museum für Tierkunde in Dresden*, 39 (2), 39–49.

Gu, X.M., Wang, H.,Chen, R.R.,Tian, Y.Z. & Li, S. (2011) The phylogenetic relationships of Paramesotriton (Caudata: Salamandridae) based on partial mitochondrial DNA gene sequences. *Zootaxa*, 2060, 59–68.

Hillis, D.M., Chamberlain, D.A., Wilcox, T.P. & Chippindale, P.T. (2001) A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea*: *Typhlomolge*) from Austin, Texas, and a systematic revision of Central Texas paedomorphic salamanders. *Herpetologica*, 57(3), 266–280.

Li ,S., Tian, Y.Z. & Gu, X.M. (2008a) A New Species of *Paramesotriton* the genus (Caudata: Salamandridae). *Acta Zootaxonomica Sinica*, 33(2), 410–413. (in Chinese)

Li, S., Tian, Y.Z., Gu, X.M. & Xiong, R.C. (2008b) A new species of *Paramesotriton — Paramesotriton longliensis* (Caudata: Salamandridae)**.** *Zoological Research*, 29(3), 313–317. (in Chinese)

Pang, J.Q., Jiang, Y.M., & Hu, Q.X. (1992) A systematic study on the Chinese newt genus *Paramesotriton* (Caudata: Salamandridae). In: *Collected Papers on Herpetology*, Jiang, Y. (ed.). Sichuan Science and Technology Publishing House, Chengdu, China, pp. 89–100. (in Chinese)

Sparreboom, M. (1983) On the sexual behaviour of *Paramesotriton caudopunctatus* (Liu and Hu) (Amphibia, Caudata, Salamandridae). *Amphidia Reptilia*, 4, 25–33.

Stuart, B.L. & Papenfuss, T.J. (2002) A new salamander of the genus *Paramesotriton* (Caudata: Salamandridae) from Laos. *Journal of Herpetology*, 36, 145–148.

Stuart, B.L.,Phimmachak, S., Sivongxay, N. & Robichaud, W.G. (2010). A new species in the *Tylototriton asperrimus* group (Caudata: Salamandridae) from central Laos. *Zootaxa*, 26–50.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F & Higgins, D.G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882.

Vieites, D.R., Min, M.-S. & Wake, D.B. (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proceedings of the National Academy of Sciences*, 104, 19903–19907.

Weisrock, D.W., Papenfuss, T.J., Macey, J.R., Litvinchuk, S.N, Polymeni, R., Ugurtas, I.H, Zhao, E.M., Jowkar, H & Larson, A.(2006) A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). *Molecular Phylogenetics and Evolution*, 41, 368–383.

Wu, Y., Rovito, S.M., Papenfuss, T.J & Hanken, J. (2009) A new species of the genus *Paramesotriton* (Caudata: Salamandridae) from Guangxi Zhuang Autonomous Region, southern China. *Zootaxa*, 2060, 59–68.

Wu, Y., Jing, K & Hanken, J. (2010) A new species of newt of the genus *Paramesotriton* (Salamandridae) from southwestern Guangdong, China, with a new northern record of *P. longliensis* from western Hubei. *Zootaxa*, 2494, 45–48.

Zhang, P., Papenfuss, T.J., Wake, M.H., Qu, L & Wake, D.B. (2008) Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution*, 49, 586–597.